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Detection Rates of TT Virus Among Children Who Visited a General Hospital in Japan

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Recently, genomic DNA of the novel TT virus (TTV) was isolated from patients suffering from posttransfusion hepatitis of unknown etiology. We examined sera from 197 children who visited the Department of Pediatrics at Toyohashi National Hospital. Sera were tested for TTV DNA by seminested polymerase chain reaction (PCR) using a set of primers synthesized according to the published TTV sequence. Ten children were found to be positive for TTV (5.1%). All positive PCR products were directly sequenced in both directions using a fluorescent dye terminator cycle sequencing system. The sequences were compared by a multiple sequence alignment and a phylogenetic tree was constructed. The phylogenetic tree showed that two of the TTV isolates found in the present experiment did not belong to any of the phylogenetic groups previously reported. ***J. Med. Virol. 57:405–407, 1999.***

KEY WORDS: DNA virus; polymerase chain reaction; phylogenetic analysis

INTRODUCTION

Recently, genomic DNA of the novel virus, TT Virus, was isolated from patients with posttransfusion hepatitis of unknown etiology [Nishizawa et al., 1997]. TTV is thought to be an unenveloped, single-strand DNA virus, and the prevalence of TTV in various populations has been studied [Okamoto et al., 1998]. However, little is known about the prevalence of TTV among Japanese children. In the present study, we investigated the prevalence of TTV genomes in children who visited the Department of Pediatrics at Toyohashi National Hospital.

MATERIALS AND METHODS

We examined sera from 197 children (age 0 month to 10 years old; 94 males, 103 females; mean age, 3.3 ± 3.4 years) who visited the Toyohashi National Hospital. Most of the children were diagnosed with a respiratory tract infection, digestive tract infection, or bronchial asthma at sampling time. None had a history of transfusions.

DNA was extracted from 100 μl of the serum obtained from each patient using the phenol-chroloformiosamyl alcohol procedure described previously [Miyake et al., 1996]. Seminested PCR was performed using a set of primers synthesized according to the published TTV sequence [Okamoto et al., 1998]. For amplification, the sense primer sequences used for first- and second-round PCR were NG059, 5′-ACAGACAGAGGAGAAGGCAACATG-3′, and NG061, 5′-GGCAACATGTTATGGATAGACTGG-3′; and the antisense primer sequence was NG063, 5′-CTGGCATTTTACCATTTCCAAAGTT-3′. PCR was performed on 20-μl aliquots of sample using Taq polymerase (Takara, Otsu, Japan) for 35 cycles, each of which consisted of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 2 min at 72°C (with an additional 7-min extension during the last cycle). Seminested PCR was performed for 30 cycles under the same conditions using 2 μl of a 10×-diluted solution of the first-round PCR products. The amplified products were resolved by electrophoresis on 3% agarose gels, stained with ethidium bromide, then observed under UV light. Sera were determined to be TTV-positive if a 271-bp band was detected. These bands were gel-purified and directly sequenced in both directions by fluorescent dye terminator cycle sequencing, using NG061 and NG063 as the sense and antisense primers, respectively, as well as a DNA sequencing kit (Perkin Elmer, Foster City, CA) and a DNA sequencer (Model 373A, Applied Biosystems, Foster City, CA). The sequences were compared by multiple sequence alignments and a phylogenetic tree was constructed using the unweighted pair group method with arithmetic mean (UPGMA). Genetic distances were calculated using ODEN version 1.2 software for molecular evolutionary analysis [Nei et al., 1986; Ina, 1994].

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TABLE I. Diagnoses and Serum ALT Levels of TTV-Positive Children at Sampling Time

Case no.	Age (year)	Sex	Diagnosis at sampling time	ALT (IU/L)[a]
1	6.6	M	Nephrosis	1
2	2.5	M	Mucocutaneous lymph node syndrome	5
3	5.4	F	Aseptic meningitis	19
4	0.9	F	Respiratory infection	14
5	2.4	M	Bronchial asthma	15
6	1.1	F	Bronchial asthma	3
7	1.5	M	Bronchiolitis	7
8	5.8	F	Urticaria	3
9	1.1	F	Failure to thrive	28
10	4.5	M	Infectious colitis	10

[a]Serum ALT levels were determined using Liquiteck GOT GPT kit (Roche Diagnostics, Basel, Switzerland) and expressed in international units per L.

RESULTS

Ten children were found to be positive for TTV (5.1%). Their diagnoses and alanine aminotransferase (ALT) levels at sampling time are shown in Table I. There were no significant differences in mean ALT levels (10.5 ± 8.5 and 17.9 ± 35.24 U; mean ± SD), mean age (3.2 ± 2.2 and 3.3 ± 3.5 years), or sex ratio (M:F 5:5 and 89:98) between TTV-positive and TTV-negative children, respectively. Multiple alignments of the TTV genomic sequences derived from the sera of TTV-positive children are shown in Figure 1. The phylogenetic tree is shown in Figure 2. Most of the TTV genomic sequences were related to the previously reported sequences, N22, G1a, G1b, G2a, G2b [Okamoto et al., 1998], and those submitted to GenBank (accession number AF072738, AF79538, and AF79541; genotype 1, 2, and 3) [Simmonds et al., 1998]. However, two se-

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     N22   CTAAGCAAAA AAAACATGAA CTATGACAAA CTACAAAGTA AATGCTTAAT ATCAGACCTA CCTCTATGGG CAGCAGCATA TGGATATGTA GAATTTTGTG
     G1a   .......... .......... .......... G......... .......... .......... .......... .......... .......... ..........
     G1b   .....T.... .......... A......... G.G....... .G...C..G. .G........ ..A..G.... .......... ...T...... .....C..CT
     G2a   ....CT...G .TG..TCAGT A...A..... .A........ .G..TC..G. .GA.A..A.G ..CT.G.... .CT...T... ......CAC. ....AC..CA
     G2b   ....CT...G .T.C.TCAGT A......... AC...G.... .G..TC.C.. .CA....A.G ..C..G.... .CT.T.TG.. C....TCTCC ....AC..CA
AF072738   ..T..T.... ........G. A......... G.G.....C. .G...C..G. .G........ ..A..T.... .......... ...M...T.. .....C..CT
AF079538   ....CT...G ..G..TCAC. A..CTCA... AC...G..C. .G..TC.G.. .GAGA..T.G .....G.... .C....T... C....TCAA. ....AC..CA
AF079541   G..TCT...T .TG..TCTGT G..CTCA... TC...G..C. .......... .GA..G.... .....G.... .C....TT.. .........C. ....AC..CA
     JC1   ...GTA..G. C.G..TCT.G A........G AC..GC..C. .....C.T.. .GA.A.A... .......... .TT...T... C..G..C.C. ..G.AC..C.
     JC2   ....C....G .TG..TCAC. G...TCA..G AC...G..C. .....C.C.. .GA.A..T.G .....G.... .CT...T... C.....CAC. ....AC..CA
     JC3   ....C....G .TG..TCAC. G...TCA..G AC...G..C. ....TC.C.. .GAGA..T.G .....G.... .CT...T... C.....CAC. ....AC..CA
     JC4   TGCTCT...G .CG...CC.C ..T.CGAG.T ....C.CAG. G..ATGCTG. .AA....A.T ...T...... .....TTTAT G..C..CAG. ..C.A.GT.A
     JC5   ...GTA..G. C.G..TCT.G A........G AC..GC..C. .....C.T.. .GA.A.A... .......... .TT...T... C..G..C.C. ..G.AC..C.
     JC6   .....T.... .......... A......... G.G....... .G...C..G. .G........ ..A..G.... .......... ...T...T.. .....C..CT
     JC7   TGCTCT...G .CG...CC.C ..T.CGAG.T ....C.CAG. G..ATGCTG. .AA....A.T ...T...... .....TTTAT G..C..CAG. ..C.A.GT.A
     JC8   ....CT.... .TG..TCAGC ...CTCA... AC......C. ....TC.T.. .GA.A..... ..C..G.... .C....T... C....T.AA. ....AC..CA
     JC9   .....T.... .......... A......... G.G....... .G...C..G. .G........ ..A..G.... .......... ...T...T.. A....C..CT
    JC10   .......... .......... .......... G......... ........G. .......... .......... .......... .......... ..........

     N22   CAAAAAGTAC AGGAGACCAA AACATACACA TGAATGCCAG GCTACTAATA AGAAGTCCCT TTACAGACCC ACAACTACTA GTACACACAG ACCCCACAAA
     G1a   .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     G1b   .T.....C.. .......AC. .......... .......... A......... ........T. .......... C..G...A.. .......... .......T..
     G2a   GC...GTA.. .......AC. ......G.AC AC..CTGT.. ATGTG....T .....C.... A.....TA.. T..G..G... .AC....ACA ..AG.CTT.G
     G2b   GC...GTA.. .......AC. ......G.GC AC..CTGT.. ATGTG.C..T .....C.... AC....TG.. T..GT.G... .AT....ACA .....CTC.G
AF072738   .T.....C.. .......AC. .......... .......... A..G...... .......... .......... C..G...A.. .........A ......AT..
AF079538   GC...GTA.. .......AC. ......G.AC AC..CTGT.. ATGTG.T..C .....C.... AC....TA.. ...G..GT.. .AC....ACA .T...CTC.G
AF079541   GC...GCC.. .......AC. ......G.AC AA..CTG... AG..G.T..T .....C.... .C..TA.... T..G...... .AC..T.ACA ....TCT..G
     JC1   .C..GGCC.. .......TCT ......G... ....C..... AG..G.T..C ..G..C.... AC..TACA.. T...A.GA.. .ACAC..AC. ....TCT..G
     JC2   GC...GTA.. .......TC. ......G.AC AC..CTG... ATGTG.T..C .....C.... AC....TA.. ...G..GT.. .AC....ACA .T...CTC.G
     JC3   GC...GTA.. .......TC. ......G.AC AC..CTG... ATGTG.T..C .....C.... AC....TA.. ...G..GT.. .AC....ACA .T...CTC.G
     JC4   .T..GGC.CT CCAC....CC GGAC.CAGT. AAG.G.T... AG..ACT..T .T.T.C..A. A....A.A.. CA.G..CTAC AAC.CAGACA G.A.AGACG.
     JC5   .C..GGCC.. .......TCT ......G... ....C..... AG..G.T..C ..G..C.... AC..TACA.. T...A.GA.. .ACAC..AC. ....TCT..G
     JC6   .T.....C.. .......AC. .......... .......... A......... .....C..T. .......... C..G...A.. .......... .......T..
     JC7   .T..GGC.CT CCAC....CC GGAC.CAGT. AAG.G.T... AG..ACT..T .T.T.C..A. A....A.A.. CA.G..CTAC AAC.CAGACA G.A.AGACG.
     JC8   GC...GTA.. .......AC. ......G.AC AC..CTG... ATGTG.T... ..G..C.... AC....TA.. ......GT.. .AC....ACA .....CTC.G
     JC9   .T.....C.. .......AC. .......... .......... A......... ........T. .......... C..G...A.. .......... .......T..
    JC10   .......... .........G .......... .......... .......... .......... .......... .......... .......... ..........

     N22   AGGCTTTGTT CCTTACTCTG TA
     G1a   .......... .........T ..
     G1b   .........A ..C..T...T ..
     G2a   G..A.AC..A ..C...AGCA ..
     G2b   G..A.AC..G ..C...AG.T .T
AF072738   .........A .....T...T ..
AF079538   .....AC..A ....------ --
AF079541   ...G.AC--- ---------- --
     JC1   .......A.A GTA...AGCT .T
     JC2   ...A.AC..A ..G...AGCT .T
     JC3   ...A.AC..A ..G...AGCT .T
     JC4   .....AC..A ..C..TGACT AT
     JC5   .......A.A GTA...AGCT .T
     JC6   .........A ..C..T...T ..
     JC7   .....AC..A ..C..TGACT AT
     JC8   ...A.AC..G ..C..TAGCC .G
     JC9   .........A ..C..T...T ..
    JC10   .......... .........T ..
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Fig. 1. Comparison of nucleotide sequences of TTV genomic fragments (222 bp) found in sera of Japanese children, i.e., JC1–JC10, N220, G1a, G1b, G2a, G2b, AF072738, AF079538, and AF79541.

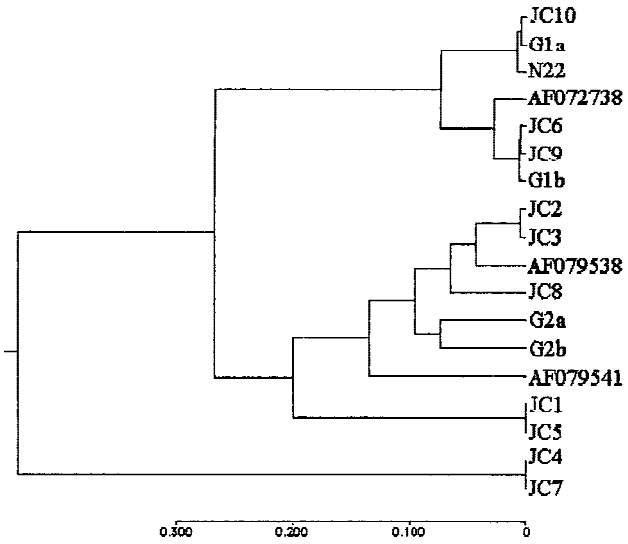

Fig. 2. Phylogenetic analysis of 15 isolates from Japanese children, i.e., JC1–JC10, N220, G1a, G1b, G2a, G2b, AF072738, AF079538, and AF79541, based on genetic distance of nucleotide sequences. Phylogenetic tree is constructed by UPGMA. Horizontal axis shows number of nucleotide substitutions per site.

quences showed a marked divergence from those previously reported.

DISCUSSION

Nishizawa et al. reported that the TTV genome was detected in sera from 3 of 5 (60%) patients suffering from posttransfusion non–A-to-G hepatitis, and 34 of 290 (12%) volunteer blood donors in Japan [Okamoto et al., 1998]. In contrast, in the United Kingdom, TTV viremia was detected in 19 (1.9%) of 1,000 nonremunerated blood donors [Simmonds et al., 1998] and 3 (10%) of 30 healthy individuals [Naoumov et al., 1998]. However, the prevalence among children has not been established to date. In the present study, we examined the prevalence of TTV genomes in the serum of children who visited the Department of Pediatrics at Toyohashi National Hospital. We detected TTV genomes in the sera of 5.1% of the children.

To date, the pathogenic role of TTV in liver disease remains unclear. Our retrospective analysis of the diagnoses of the TTV-positive children revealed no specific clinical characteristics. All showed normal ALT levels, implying that TTV viremia may not cause the elevation of serum ALT in all situations. However, in the present study, we examined only one-point serum samples for the presence of the TTV genome. Therefore, we were unable to determine whether the TTV viremia observed in these patients was transient or persistent. Thus, further studies are needed to elucidate the characteristics of TTV infection.

Phylogenetic analysis of the TTV nucleotide sequences found in a previous study revealed two major groups, G1 and G2, which were each subdivided into two subgroups, G1a and G1b, and G2a and G2b, respectively [Okamoto et al., 1998]. Another novel TTV genotype has also been described as type 3 [Simmonds et al., 1998]. However, the phylogenetic tree constructed in the present study showed two TTV isolates with high heterogeneity that did not belong to any of these groups.

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